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Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

This online publication has been corrected. The corrected version first appeared at thelancet.com on January 7, 2020.

Supplement to: Logunov DY, Dolzhikova IV, Zubkova OV, Tukhvatulin AI, Shcheblyakov DV, Dzharullaeva AS, et al. Safety and immunogenicity of an rAd26 and rAd5 vector-based heterologous prime-boost COVID-19 vaccine in two formulations: two open, non-randomised phase 1/2 studies from Russia. *Lancet* 2020; published online Sept 4. http://dx.doi.org/10.1016/S0140-6736(20)31866-3.

Appendix 1

Determination of immunogenicity

Antigen-specific humoral immune response was analyzed on days 0, 14, 21, and 28 on first stage and on days 0, 14, 21, 28, and 42 on second stage. The titer of glycoprotein-specific antibodies in serum was determined by ELISA. Anti-SARS-CoV-2 IgG ELISA test-system developed in Gamaleya NRCEM and registered for clinical use in Russian Federation (P3H 2020/10393 2020-05-18) was used. Test-system determines the IgG specific to receptor-binding domain of SARS-CoV-2 glycoprotein S. Briefly, the RBD-pre-coated plates (100 ng per well) were washed 5x with 0,1% wash solution and then blocked with blocking solution. Serum samples were serially diluted in blocking solution and added in wells, then plates were incubated at 37°C for 1 h. After washing the plates 5x the peroxidase-conjugated anti-human IgG detection antibodies diluted in blocking solution were added and plates were incubated at 37°C for 1 h. After washing 5x the substrate TMB was added and plates were incubated at 20-25°C for 15 min, the reaction was stopped with stop solution. The OD signals were determined with a spectrophotometer Multiskan FC (Thermo Fisher Scientific Inc, USA) at 450 nm. The IgG titer was determined as the maximum dilution of serum, in which the OD_{450} value of the serum of the immunized participant exceeds the value of the control serum (serum of the participant before immunization) by more than 2 times. For the study of S1-specific IgG, the recombinant S1 was adsorbed into the wells (100 ng per well) of the plate; further study was carried out as described above. When calculating the percentage of volunteers who responded to the vaccination, as all volunteers didn't have antibodies to SARS-CoV-2 on day 0 of the trials, the volunteer was considered as “responder” if the antigen-specific IgG titer was 25 and higher at days after vaccination.

Determination of neutralizing antibody (NtAb) titer was done on days 0, 14, and 28 on first stage and on days 0, 14, 28, and 42 on second stage. NtAb titer was determined by microneutralization test using SARS-CoV-2 (hCoV-19/Russia/Moscow_PMVL-1/2020) in a 96-well plate. Serum samples were inactivated by incubation at 56ºC for 30 min and serial two-fold dilutions in Dulbecco's modified eagle medium containing 2% heat-inactivated fetal bovine serum at a range 1:2·5 – 1:640 were made. In the analysis, blood serum samples with the characterized neutralizing activity are always taken as a control: positive control - with NtAb 80, negative control with NtAb <2.5 Then $100TCID_{50}$ (median tissue culture infectious dose) of SARS-CoV-2 in volume of 50 ul was added to each sample in volume 50 ul. The samples were incubated at 37ºC for 1 h, added to Vero E6 cells and incubated in a 5% CO_2 incubator at 37ºC for 96 h. The cytopathic effect (CPE) of the virus on the cell monolayer was assessed visually, if even a slight damage to the monolayer (1-2 «plaques») was observed in the well, this well was considered as a well with a manifestation of CPE. Neutralization titer was defined as the highest serum dilution without any CPE in two of three replicable wells. When calculating the percentage of volunteers who responded to the vaccination, as all volunteers didn't have antibodies to SARS-CoV-2 on day 0 of the trials, the volunteer was considered as “responder” if the NtAb titer was 5 and higher at days after vaccination.

Whole-blood samples were collected on days 0, 14, and 28 after the first injection. PBMC were isolated by Ficoll (1·077 g/mL; PanEco, Russia) density gradient centrifugation (800g for 30 min). Cells were stained with carboxyfluorescein using a succinimidyl ester (CFSE) tracer kit (Invitrogen, USA) according to the procedure described previously.[9] The cells were seeded in 96-well plates (2×10^5 cells/well), re-stimulated with the recombinant RBD at 1 μg/well. Phytohemagglutinin was used as a positive control. Media were collected 72 hours after stimulation, and analyzed for interferon-γ by ELISA (Human gamma-Interferon-ELISA-BEST, A-8752, Vector Best). Results are reported as fold increase in interferon-γ concentration upon exposure to antigen. The volunteer was considered as “responder” if the increase in the concentration of IFN-γ by antigen-restimulated PBMC after vaccination at days 14 or 28 exceeded the increase in the concentration of IFN-γ before vaccination, when calculating the percentage of responding volunteers. Cells were analyzed by flow cytometry 72 hours later on a FACSAriaIII (BD Biosciences, USA) flow cytometer with BD FACSDiva Software

(BD Biosciences, USA).[10] Proliferating CD4+ or CD8+ T lymphocytes were identified by forward and side light scatter, expression of CD3, CD4, CD8, and low fluorescence from CFSE. Proliferation in unstimulated cells were subtracted from that of stimulated cells, and negative differences were set to zero. Results are reported as percent proliferating cells. The volunteer was considered as "responder" if proliferation of antigen-restimulated T-cells after vaccination at days 14 or 28 exceeded the proliferation before vaccination, when calculating the percentage of responding volunteers.

To determine rAd-specific NtAb titers, heat-inactivated serum samples were serially diluted and 50 µl was dispensed in duplicate in 96-well plates (five serum samples per plate). Next, 50 µl of adenovirus stock (rAd26-EGFP or rAd5-EGFP), expressing fluorescent protein EGFP, diluted to 500 50% cell culture infectious doses, was added to the wells containing serum. Plates were incubated for 1 h at 37°C in 5% CO_2 before the addition of 100 µl (4×10^4 cells per well) of HEK293 cell suspension, after which plates were further incubated overnight. As a positive control for virus replication, only adenovirus (rAd26-EGFP or rAd5-EGFP) was added to cells (four wells), and as a negative control, cells were cultured in the absence of virus (four wells). Supernatant was removed three days later, and 200 µl of fresh medium was added to all wells, followed by an incubation period for another 4 days. On the day 7 of incubation, the number of EGFP fluorescent cell focuses was counted. Neutralization titers were defined as the maximum serum dilution where 50% reduction of EGFP fluorescent cell focuses compared with the positive control was determined.

Table S1. Changes in laboratory parameteres, systemic and local adverse events after vaccination with rAd26-S or rAd5-S on the first stage. The table shows the total number of volunteers with developed AEs, as well as the number of volunteers with developed AEs according to the severity of AEs (mild, moderate, and severe). If the severity is not indicated, the severity was mild. Grade 3 AEs were not detected through all studies.

MedDRA "System Organ Classes" (SOCs)	Number of participants with AEs (%)			
	Gam-COVID-Vac		Gam-COVID-Vac-Lyo	
	rAd26-S (n=9)	rAd5-S (n=9)	rAd26-S (n=9)	rAd5-S (n=9)
SOC Investigations				
Aspartate aminotransferase increased	1 (11·1)	0	0	0
Mild (Grade 1)	0	0	0	0
Moderate (Grade 2)	1 (11·1)	0	0	0
Blood cholesterol increased	1 (11·1)	0	0	0
Blood immunoglobulin A increased	0	0	0	1 (11·1)
Blood immunoglobulin M decreased	1 (11·1)	1 (11·1)	0	0
Blood immunoglobulin E increased	1 (11·1)	2	0	0
Blood lactate dehydrogenase increased	0	1 (11·1)	0	0
Blood pressure increased	1 (11·1)	0	0	0
Blood pressure decreased	1 (11·1)	0	0	0
B-lymphocyte count decreased	0	0	0	3 (33·3)
B-lymphocyte count increased	3 (33·3)	5 (55·6)	2 (22·2)	0
CD4 lymphocytes increased	2 (22·2)	2 (22·2)	1 (11·1)	4 (44·4)
CD4 lymphocytes decreased	1 (11·1)	0	0	0
CD4 lymphocytes decreased	0	0	0	0
CD4/CD8 ratio decreased	1 (11·1)	1 (11·1)	1 (11·1)	0
CD4/CD8 ratio increased	0	1 (11·1)	0	0
CD8 lymphocyte percentage decreased	4 (44·4)	0	0	0
CD8 lymphocytes decreased	1 (11·1)	0	0	0
CD8 lymphocytes increased	1 (11·1)	2 (22·2)	1 (11·1)	0
Lymphocyte count increased	0	0	2 (22·2)	0
Lymphocyte percentage increased	1 (11·1)	0	0	0
Natural killer cell count decreased	5 (55·6)	4 (44·4)	6 (66·7)	4 (44·4)

Natural killer cell count increased	0	2 (22·2)	0	0
Monocyte count increased	5 (55·6)	0	0	0
Platelet count increased	1 (11·1)	0	0	0
Red blood cell sedimentation rate increased	1 (11·1)	1 (11·1)	0	0
T-lymphocyte count increased	3 (33·3)	6 (66·7)	2 (22·2)	4 (44·4)
T-lymphocyte count decreased	1 (11·1)	0	0	0
White blood cell count increased	1 (11·1)	0	0	0
SOC Nervous system disorders				
Headache	6 (66·7)	3 (33·3)	3 (33·3)	4 (44·4)
SOC General disorders and administration site conditions				
Administration site induration	0	0	0	1 (11·1)
Asthenia	3 (33·3)	3 (33·3)	0	0
Decreased appetite	2 (22·2)	0	0	0
Hyperthermia	8 (88·9)	2 (22·2)	1 (11·1)	1 (11·1)
Pain (muscle and joint pain)	3 (33·3)	2 (22·2)	1 (11·1)	2 (22·2)
Pyrexia	0	1 (11·1)	0	0
Mild (Grade 1)	0	0	0	0
Moderate (Grade 2)	0	1 (11·1)	0	0
Vaccination site pain	7 (77·8)	5 (55·6)	5 (55·6)	7 (77·8)
Vaccination site pruritus	1 (11·1)	0	0	0
Vaccination site warmth	0	0	0	1 (11·1)
SOC Respiratory, thoracic and mediastinal disorders				
Oropharyngeal pain	0	1 (11·1)	0	0
SOC Gastrointestinal disorders				
Diarrhoea	1 (11·1)	0	0	0
SOC Cardiac disorders				
Palpitations	3 (33·3)	1 (11·1)	0	0
SOC Immune system disorders				
Urticaria	1 (11·1)	0	0	0

Table S2. Changes in laboratory parameteres, systemic and local adverse events after vaccination with rAd26-S and rAd5-S on the second stage. The table shows the total number of volunteers with developed AEs, as well as the number of volunteers with developed AEs according to the severity of AEs (mild, moderate, and severe). If the severity is not indicated, the severity was mild. The table shows total number of participants with developed AEs throughout the study, as well as number of participants with AEs after each vaccination. Grade 3 AEs were not detected through all studies.

MedDRA "System Organ Classes" (SOCs)	Number of participants with AEs (%)					
	Gam-COVID-Vac			Gam-COVID-Vac-Lyo		
	Total number rAd26-S + rAd5-S (n=20)	After rAd26-S till 21 day (n=20)	After rAd5-S from day 21 till day 42 (n=20)	Total number rAd26-S + rAd5-S (n=20)	After rAd26-S till 21 day (n=20)	After rAd5-S from day 21 till day 42 (n=20)
SOC Investigations						
Alanine aminotransferase increased	0	0	0	1 (5)	0	1 (5)
Blood bilirubin increased	0	0	0	1 (5)	0	1 (5)
Blood cholesterol increased	0	0	0	1 (5)	0	1 (5)
Blood creatinine decreased	0	0	0	1 (5)	0	1 (5)
Blood immunoglobulin A increased	1 (5)	0	1 (5)	0	0	0
Blood immunoglobulin E decreased	0	0	0	1 (5)	0	1 (5)
Blood immunoglobulin E increased	3 (15)	1 (5)	2 (10)	4 (20)	0	4 (20)
Blood lactate dehydrogenase decreased	0	0	0	1 (5)	0	1 (5)
B-lymphocyte count decreased	5 (25)	0	5 (25)	0	0	0
B-lymphocyte count increased	4 (20)	0	4 (20)	7 (35)	0	7 (35)
CD4 lymphocytes increased	6 (30)	0	6 (30)	8 (40)	0	8 (40)
CD4 lymphocytes decreased	2 (10)	0	2 (10)	0	0	0
CD4 lymphocyte percentage decreased	3 (15)	0	3 (15)	0	0	0
CD4 lymphocytes decreased	2 (10)	0	2 (10)	0	0	0
CD4/CD8 ratio decreased	4 (20)	0	4 (20)	2 (10)	0	2 (10)
CD4/CD8 ratio increased	2 (10)	0	2 (10)	0	0	0
CD8 lymphocytes increased	3 (15)	0	3 (15)	6 (30)	0	6 (30)
Haematocrit decreased	3 (15)	3 (15)	0	0	0	0
Lymphocyte count increased	3 (15)	2 (10)	1 (5)	7 (35)	0	7 (35)
Lymphocyte percentage increased	7 (35)	0	7 (35)	0	0	0

Natural killer cell count decreased	7 (35)	0	7 (35)	6 (30)	0	6 (30)
Natural killer cell count increased	4 (20)	0	4 (20)	1 (5)	0	1 (5)
Neutrophil count decreased	1 (5)	0	1 (5)	0	0	0
Monocyte count increased	1 (5)	0	1 (5)	0	0	0
Platelet count decreased	1 (5)	0	1 (5)	0	0	0
Platelet count increased	1 (5)	1 (5)	0	0	0	0
Red blood cell in urine	2 (10)	2 (10)	0	0	0	0
Red blood cell sedimentation rate increased	2 (10)	0	2 (10)	0	0	0
T-lymphocyte count increased	7 (35)	0	7 (35)	10 (50)	0	10 (50)
White blood cell count increased	1 (5)	0	1 (5)	0	0	0
SOC Nervous system disorders						
Headache	11 (55)	9 (45)	5 (25)	5 (25)	4 (20)	2 (10)
Mild (Grade 1)	9 (45)	7 (35)	5 (25)	5 (25)	4 (20)	2 (10)
Moderate (Grade 2)	2 (10)	2 (10)	0	0	0	0
SOC General disorders and administration site conditions						
Asthenia	11 (55)	6 (30)	7 (35)	4 (20)	0	4 (20)
Chills	2 (10)	0	2 (10)	0	0	0
Decreased appetite	1 (5)	2 (10)	0	0	0	0
Hyperthermia	19 (95)	18 (80)	8 (40)	7 (35)	5 (25)	2 (10)
Malaise	2 (10)	0	2 (10)	0	0	0
Pain (muscle and joint pain)	4 (20)	3 (15)	4 (20)	6 (30)	1 (5)	6 (30)
Mild (Grade 1)	4 (20)	2 (10)	4 (20)	4 (20)	0	4 (20)
Moderate (Grade 2)	1 (5)	1 (5)	0	2 (10)	1 (5)	2 (10)
Pyrexia	1 (5)	0	1 (5)	0	0	0
Mild (Grade 1)	0	0	0	0	0	0
Moderate (Grade 2)	1 (5)	0	1 (5)	0	0	0
Vaccination site edema	1 (5)	1 (5)	0	0	0	0
Vaccination site pain	8 (40)	6 (30)	4 (20)	12 (60)	8 (40)	11 (55)
Vaccination site warmth	2 (10)	0	2 (10)	0	0	0

SOC Respiratory, thoracic and mediastinal disorders						
Nasal stuffiness	1 (5)	0	1 (5)	0	0	0
Oropharyngeal pain	1 (5)	0	1 (5)	0	0	0
Rhinorrhea	4 (20)	1 (5)	3 (15)	0	0	0
Sneezing	1 (5)	0	1 (5)	0	0	0
Throat clearing	2 (10)	1 (5)	1 (5)	0	0	0
SOC Gastrointestinal disorders						
Diarrhoea	3 (15)	2 (10)	1 (5)	0	0	0

Table S3. Seroconversion rate and statistic data (median, 25% and 75% percentile, geometric mean and 95% CI of geometric mean) of RBD-specific antibodies at days 0, 14, 21, and 28, as measured by ELISA, in participants immunized with rAd26-S (n=9) or rAd5-S (n=9) only. Titer of 12·5 is a baseline characteristic that correspond to volunteers “non-responder”.

	Gam-COVID-Vac								Gam-COVID-Vac-Lyo							
	rAd26-S				rAd5-S				rAd26-S				rAd5-S			
	0 day	14 day	21 day	28 day	0 day	14 day	21 day	28 day	0 day	14 day	21 day	28 day	0 day	14 day	21 day	28 day
Seroconversion rate, %	0	100	100	100	0	77·8	100	100	0	77·8	100	100	0	88·9	100	100
25% Percentile	12·5	250·0	1200	1200	12·5	18·75	800·0	800·0	12·5	18·75	400·0	600·0	12·5	25·00	1000	1200
Median	12·5	400·0	1600	1600	12·5	200·0	1600	1600	12·5	25·00	800·0	1600	12·5	400·0	3200	3200
75% Percentile	12·5	1200	3200	3200	12·5	1000	4800	6400	12·5	150·0	2400	2400	12·5	800·0	6400	6400
Geometric mean	12·5	400·0	1866	1866	12·5	147·0	2177	2352	12·5	46·29	1089	1372	12·5	158·7	2016	2963
Lower 95% CI of geo. mean	12·5	142·6	1112	1112	12·5	26·09	1020	1052	12·5	19·57	509·8	685·5	12·5	39·77	532·1	1366
Upper 95% CI of geo. mean	12·5	1122	3132	3132	12·5	828·0	4650	5255	12·5	109·5	2325	2744	12·5	633·7	7637	6426

Table S4. Seroconversion rate and statistic data (median, 25% and 75% percentile, geometric mean and 95% CI of geometric mean) of RBD-specific antibodies in participants immunized with rAd26-S and rAd5-S (n=20) at days 0, 14, 21, 28, and 42 and in COVID-19 convalescents at ~1 month after recovery as measured by ELISA. Titer of 12·5 is a baseline characteristic that correspond to volunteers “non-responder”.

	Gam-COVID-Vac (rAd26-S + rAd5-S)					Gam-COVID-Vac-Lyo (rAd26-S + rAd5-S)						Convalescents
	0 day	14 day	21 day	28 day	42 day	0 day	14 day	21 day	28 day	42 day		
Seroconversion rate, %	0	95	100	100	100	0	75	100	100	100		85·8
25% Percentile	12·5	50·00	500·0	3200	6400	12·5	15·63	350·0	2000	6400		400
Median	12·5	100·0	1600	6400	25600	12·5	50·00	1200	6400	12800		3200
75% Percentile	12·5	350·0	3200	12800	25600	12·5	100·0	3200	12800	25600		6400
Geometric mean	12·5	132·0	1345	5382	14703	12·5	50·00	951·4	5322	11143		1266
Lower 95% CI of geo. mean	12·5	69·32	756·9	3538	9576	12·5	28·37	485·4	3184	7786		1066
Upper 95% CI of geo. mean	12·5	251·2	2392	8185	22576	12·5	88·13	1865	8894	15947		1504

Table S5. Seroconversion rate and statistic data (median, 25% and 75% percentile, geometric mean and 95% CI of geometric mean) of NtAb at days 0, 14, and 28, as measured by microneutralization assay, in participants immunized with rAd26-S (n=9) or rAd5-S (n=9) only. Titer of 1·25 is a baseline characteristic that correspond to volunteers “non-responder”.

	Gam-COVID-Vac						Gam-COVID-Vac-Lyo					
	rAd26-S			rAd5-S			rAd26-S			rAd5-S		
	0 day	14 day	28 day	0 day	14 day	28 day	0 day	14 day	28 day	0 day	14 day	28 day
Seroconversion rate, %	0	55·6	66·7	0	55·6	66·7	0	22·2	55·6	0	66·7	88·9
25% Percentile	1·250	1·250	1·250	1·250	1·250	1·250	1·250	1·250	1·250	1·250	1·250	5·000
Median	1·250	5·000	5·000	1·250	5·000	10·00	1·250	1	5·000	1·25	5·000	20·00
75% Percentile	1·250	7·500	10·00	1·250	10·00	15·00	1·250	3·125	10·00	1·25	10·00	30·00
Geometric mean	1·250	3·402	4·286	1·250	4·286	6·300	1·250	1·984	3·674	1·25	4·286	10·80
Lower 95% CI of geo. mean	1·250	1·522	1·947	1·250	1·439	2·325	1·250	0·9340	1·575	1·25	2·039	4·386
Upper 95% CI of geo. mean	1·250	7·602	9·437	1·250	12·76	17·07	1·250	4·215	8·572	1·25	9·010	26·60

Table S6. Seroconversion rate and statistic data (median, 25% and 75% percentile, geometric mean and 95% CI of geometric mean) of NtAb n participants immunized with rAd26-S and rAd5-S (n=20) at days 0, 14, 28, and 42 and in COVID-19 convalescents at ~1 month after recovery, as measured by microneutralization assay. Titer of 1·25 is a baseline characteristic that correspond to volunteers “non-responder”.

	Gam-COVID-Vac (rAd26-S + rAd5-S)				Gam-COVID-Vac-Lyo (rAd26-S + rAd5-S)				
	0 day	14 day	28 day	42 day	0 day	14 day	28 day	42 day	Convalescent
Seroconversion rate, %	0	65	95	100	0	25	95	100	90·9
25% Percentile	1·250	1·250	10·00	20·00	1·250	1·250	12·50	40·00	20·00
Median	1·250	5·000	20·00	40·00	1·250	1·250	20·00	40·00	40·00
75% Percentile	1·250	17·50	40·00	80·00	1·250	4·063	40·00	80·00	80·00
Geometric mean	1·250	4·830	16·25	49·25	1·250	1·961	21·44	45·95	32·96
Lower 95% CI of geo. mean	1·250	2·782	10·38	33·17	1·250	1·337	13·91	32·11	31·49
Upper 95% CI of geo. mean	1·250	8·385	25·42	73·12	1·250	2·878	33·04	65·76	34·50

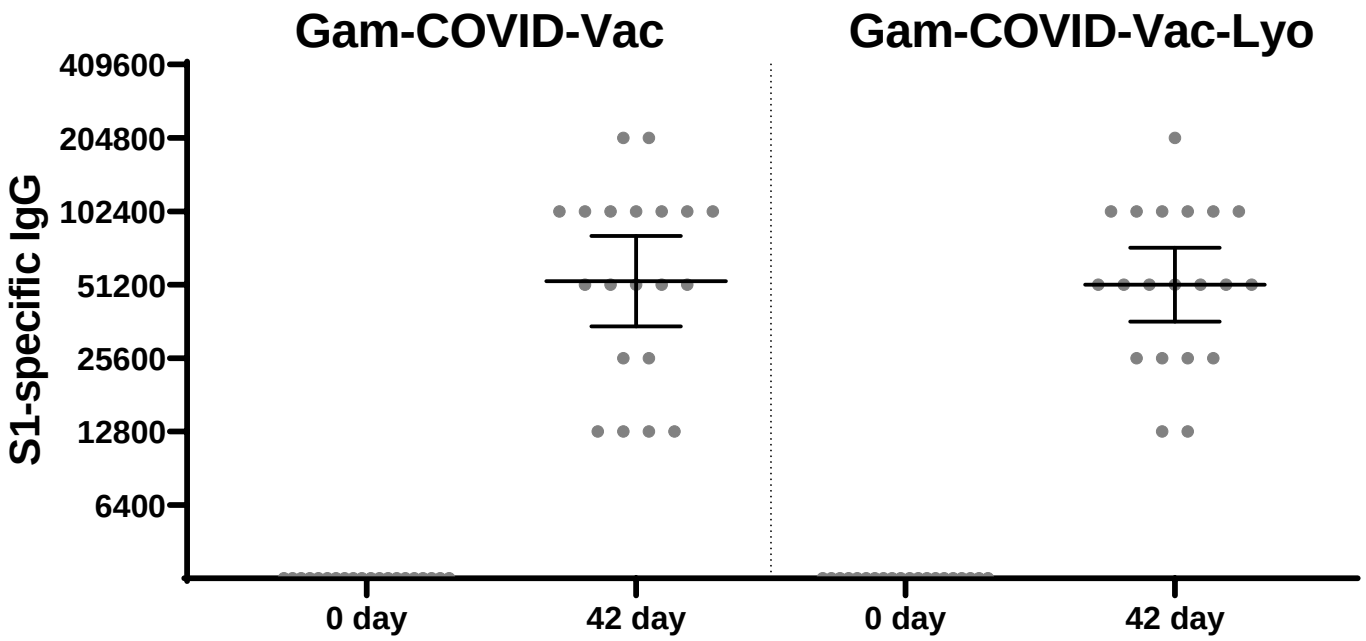
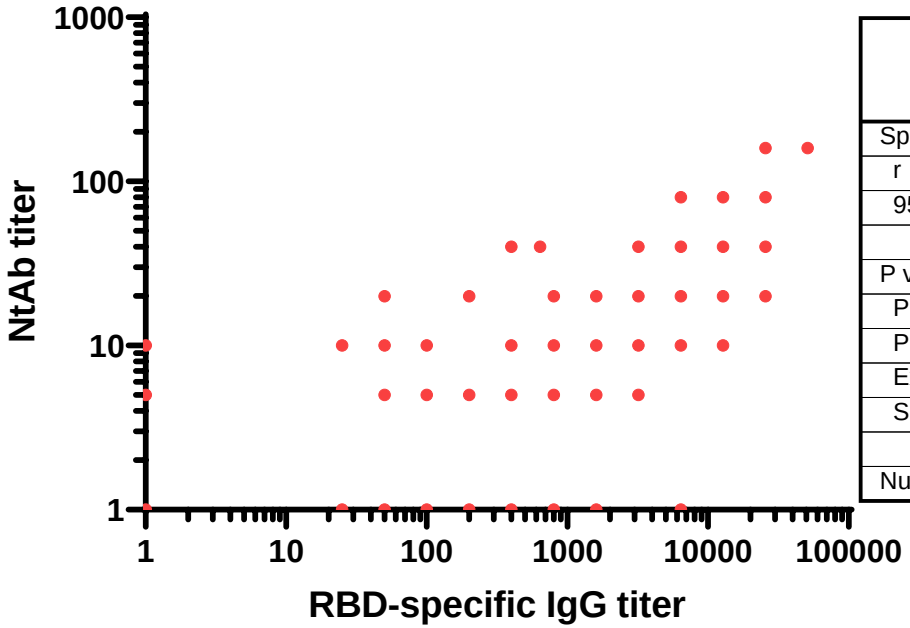


Figure S1. Glycoprotein S1-specific antibodies at days 0 and 42, as measured by ELISA, in participants immunized with with rAd26-S and rAd5-S (n=20) with 21 days interval



	RBD ELISA vs. NtAb
Spearman r	
r	0.8197
95% confidence interval	0.7654 to 0.8624
P value	
P (two-tailed)	<0.0001
P value summary	****
Exact or approximate P value?	Approximate
Significant? (alpha = 0.05)	Yes
Number of XY Pairs	192

Figure S2. Correlation of RBD-specific IgG in serum samples of immunized volunteers with NtAb titers.

Table S7. Participants with detected CD4+ and CD8+ proliferative cellular immune response.

Group		Number and (%) of participants with detected immune response at day from first administration		
		14 day	28 day	14 and/or 28 day
Gam-COVID-Vac				
rAd26-S (n=9)	CD4+	3 (33·33)	6 (66·67)	7 (77·78)
	CD8+	2 (22·22)	6 (66·67)	7 (77·78)
	CD4+ and/or CD8+	3 (33·33)	6 (66·67)	7 (77·78)
rAd5-S (n=9)	CD4+	1 (11·11)	8 (88·89)	8 (88·89)
	CD8+	1 (11·11)	8 (88·89)	8 (88·89)
	CD4+ and/or CD8+	1 (11·11)	8 (88·89)	8 (88·89)
rAd26-S + rAd5-S (n=20)	CD4+	11 (55·00)	17 (85·00)	17 (85·00)
	CD8+	13 (65·00)	17 (85·00)	18 (90·00)
	CD4+ and/or CD8+	14 (70·00)	18 (90·00)	19 (95·00)
Gam-COVID-Vac-Lyo				
rAd26-S (n=9)	CD4+	2 (22·22)	6 (66·67)	7 (77·78)
	CD8+	2 (22·22)	6 (66·67)	7 (77·78)
	CD4+ and/or CD8+	2 (22·22)	6 (66·67)	7 (77·78)
rAd5-S (n=9)	CD4+	2 (22·22)	6 (66·67)	6 (66·67)
	CD8+	2 (22·22)	6 (66·67)	7 (77·78)
	CD4+ and/or CD8+	3 (33·33)	6 (66·67)	7 (77·78)
rAd26-S + rAd5-S (n=20)	CD4+	12 (60·00)	20 (100·00)	**20 (100·00)**
	CD8+	12 (60·00)	20 (100·00)	**20 (100·00)**
	CD4+ and/or CD8+	12 (60·00)	20 (100·00)	**20 (100·00)**

Table S8. Participants with detected IFNgamma cellular immune response.

Group	Number and (%) of participants with detected immune response at day from first administration		
	14 day	28 day	14 and/or 28 day
Gam-COVID-Vac			
rAd26-S (n=9)	9 (100·00)	8 (88·89)	**9 (100·00)**
rAd5-S (n=9)	8 (88·89)	8 (88·89)	**9 (100·00)**
rAd26-S + rAd5-S (n=20)	16 (80·00)	18 (90·00)	**20 (100·00)**
Gam-COVID-Vac-Lyo			
rAd26-S (n=9)	6 (66·67)	6 (66·67)	6 (66·67)
rAd5-S (n=9)	7 (77·78)	9 (100·00)	**9 (100·00)**
rAd26-S + rAd5-S (n=20)	13 (65·00)	17 (85·00)	**20 (100·00)**

Table S9. Statistic data (median, 25% and 75% percentile, 95%CI, Mean, SD, SEM) of antigen-specific CD4+ cell proliferation at days 0, 14, and 28, as measured by flow cytometry, in participants immunized with rAd26-S (n=9) or rAd5-S (n=9) only

	Gam-COVID-Vac						Gam-COVID-Vac-Lyo					
	rAd26-S			rAd5-S			rAd26-S			rAd5-S		
	0 day	14 day	28 day	0 day	14 day	28 day	0 day	14 day	28 day	0 day	14 day	28 day
25% Percentile	0·000	0·000	0·000	0·000	0·000	0·6000	0·000	0·000	0·000	0·000	0·000	0·000
Median	0·000	0·000	0·6000	0·000	0·000	1·100	0·000	0·000	0·2000	0·000	0·000	1·600
75% Percentile	0·1000	0·1500	1·450	0·1000	0·1000	2·750	0·000	0·1000	2·600	0·000	0·1500	2·350
95% CI of median												
Actual confidence level	96·09%	96·09%	96·09%	96·09%	96·09%	96·09%	96·09%	96·09%	96·09%	96·09%	96·09%	96·09%
Lower confidence limit	0·000	0·000	0·000	0·000	0·000	0·6000	0·000	0·000	0·000	0·000	0·000	0·000
Upper confidence limit	0·1000	0·2000	1·800	0·2000	0·2000	3·500	0·000	0·2000	2·600	0·000	0·2000	2·800
Mean	0·04444	0·1000	1·178	0·04444	0·08889	1·744	0·01111	0·06667	1·167	0·01111	0·07778	1·544
Std. Deviation	0·07265	0·2000	1·839	0·08819	0·2028	1·695	0·03333	0·1414	1·304	0·03333	0·1394	1·728
Std. Error of Mean	0·02422	0·06667	0·6130	0·02940	0·06759	0·5650	0·01111	0·04714	0·4346	0·01111	0·04648	0·5759

Table S10. Statistic data (median, 25% and 75% percentile, 95%CI, Mean, SD, SEM) of antigen-specific CD8+ cell proliferation at days 0, 14, and 28, as measured by flow cytometry, in participants immunized with rAd26-S (n=9) or rAd5-S (n=9) only

	Gam-COVID-Vac						Gam-COVID-Vac-Lyo					
	rAd26-S			rAd5-S			rAd26-S			rAd5-S		
	0 day	14 day	28 day	0 day	14 day	28 day	0 day	14 day	28 day	0 day	14 day	28 day
25% Percentile	0·000	0·000	0·000	0·000	0·000	0·4000	0·000	0·000	0·000	0·000	0·000	0·000
Median	0·000	0·000	0·3000	0·000	0·000	1·400	0·000	0·000	0·4000	0·000	0·000	0·8000
75% Percentile	0·1000	0·1000	1·000	0·05000	0·000	2·050	0·000	0·1000	2·100	0·05000	0·2000	1·900
95% CI of median												
Actual confidence level	96·09%	96·09%	96·09%	96·09%	96·09%	96·09%	96·09%	96·09%	96·09%	96·09%	96·09%	96·09%
Lower confidence limit	0·000	0·000	0·000	0·000	0·000	0·3000	0·000	0·000	0·000	0·000	0·000	0·000
Upper confidence limit	0·1000	0·2000	1·000	0·1000	0·000	2·100	0·000	0·2000	2·100	0·1000	0·3000	2·300
Mean	0·04444	0·1000	0·7333	0·02222	0·06667	1·400	0·01111	0·05556	0·9222	0·02222	0·08889	1·256
Std. Deviation	0·07265	0·2345	1·118	0·04410	0·2000	1·100	0·03333	0·1130	1·011	0·04410	0·1269	1·595
Std. Error of Mean	0·02422	0·07817	0·3727	0·01470	0·06667	0·3667	0·01111	0·03768	0·3370	0·01470	0·04231	0·5315

Table S11. Statistic data (median, 25% and 75% percentile, 95%CI, Mean, SD, SEM) of antigen-specific PBMC cell IFNgamma production at days 0, 14, and 28, as measured by ELISA, in participants immunized with rAd26-S (n=9) or rAd5-S (n=9) only

	Gam-COVID-Vac						Gam-COVID-Vac-Lyo					
	rAd26-S			rAd5-S			rAd26-S			rAd5-S		
	0 day	14 day	28 day	0 day	14 day	28 day	0 day	14 day	28 day	0 day	14 day	28 day
25% Percentile	1·021	2·726	2·348	1·000	2·470	2·304	1·048	1·630	1·671	1·000	2·886	14·46
Median	1·083	4·045	4·580	1·041	5·316	5·792	1·142	2·762	6·282	1·068	5·609	19·47
75% Percentile	1·300	5·432	10·88	1·144	12·73	30·46	1·209	4·371	47·63	1·547	15·23	325·7
95% CI of median												
Actual confidence level	96·09%	96·09%	96·09%	96·09%	96·09%	96·09%	96·09%	96·09%	96·09%	96·09%	96·09%	96·09%
Lower confidence limit	1·000	2·623	2·007	1·000	2·267	2·005	1·000	1·381	1·424	1·000	2·665	11·26
Upper confidence limit	1·328	6·597	13·94	1·148	13·54	42·92	1·242	4·855	73·57	1·602	15·50	522·3
Mean	1·142	4·442	6·434	1·123	6·967	46·53	1·150	3·028	48·94	1·412	11·37	213·7
Std. Deviation	0·1491	2·419	4·914	0·2365	5·083	105·0	0·1236	1·413	104·7	0·7843	13·23	392·0
Std. Error of Mean	0·04970	0·8063	1·638	0·07884	1·694	35·01	0·04120	0·4711	34·90	0·2614	4·411	130·7

Table S12. Statistic data (median, 25% and 75% percentile, 95%CI, Mean, SD, SEM) of antigen-specific CD4+ cell proliferation at days 0, 14, and 28, as measured by flow cytometry, in participants immunized immunized with rAd26-S and rAd5-S (n=20)

	Gam-COVID-Vac			Gam-COVID-Vac-Lyo		
	0 day	14 day	28 day	0 day	14 day	28 day
25% Percentile	0·000	0·000	0·2000	0·000	0·000	0·3250
Median	0·000	0·2500	2·500	0·000	0·2000	1·300
75% Percentile	0·07500	1·625	3·275	0·000	0·8500	2·275
95% CI of median						
Actual confidence level	95·86%	95·86%	95·86%	95·86%	95·86%	95·86%
Lower confidence limit	0·000	0·000	0·2000	0·000	0·000	0·4000
Upper confidence limit	0·000	1·400	3·200	0·000	0·7000	2·200
Mean	0·03000	1·645	2·265	0·02000	0·8100	1·765
Std. Deviation	0·05712	3·259	2·075	0·05231	1·456	1·922
Std. Error of Mean	0·01277	0·7286	0·4639	0·01170	0·3256	0·4298

Table S13. Statistic data (median, 25% and 75% percentile, 95%CI, Mean, SD, SEM) of antigen-specific CD8+ cell proliferation at days 0, 14, and 28, as measured by flow cytometry, in participants immunized immunized with rAd26-S and rAd5-S (n=20)

	Gam-COVID-Vac			Gam-COVID-Vac-Lyo		
	0 day	14 day	28 day	0 day	14 day	28 day
25% Percentile	0·000	0·000	0·2000	0·000	0·000	0·4000
Median	0·000	0·1500	1·300	0·000	0·3000	1·100
75% Percentile	0·000	1·350	3·500	0·000	0·8250	2·300
95% CI of median						
Actual confidence level	95·86%	95·86%	95·86%	95·86%	95·86%	95·86%
Lower confidence limit	0·000	0·000	0·2000	0·000	0·000	0·4000
Upper confidence limit	0·000	1·200	2·600	0·000	0·6000	2·000
Mean	0·02500	1·515	2·080	0·01000	0·6900	1·615
Std. Deviation	0·05501	3·080	2·431	0·03078	1·208	1·807
Std. Error of Mean	0·01230	0·6888	0·5436	0·006882	0·2701	0·4041

Table S14. Statistic data (median, 25% and 75% percentile, 95%CI, Mean, SD, SEM) of antigen-specific PBMC cell IFNgamma production at days 0, 14, and 28, as measured by ELISA, in participants immunized immunized with rAd26-S and rAd5-S (n=20)

	Gam-COVID-Vac			Gam-COVID-Vac-Lyo		
	0 day	14 day	28 day	0 day	14 day	28 day
25% Percentile	1·000	2·896	5·393	1·000	1·342	2·621
Median	1·036	4·474	12·72	1·097	2·630	5·706
75% Percentile	1·118	9·617	41·94	1·168	5·922	22·71
95% CI of median						
Actual confidence level	95·86%	95·86%	95·86%	95·86%	95·86%	95·86%
Lower confidence limit	1·000	2·929	6·310	1·000	1·404	2·972
Upper confidence limit	1·105	7·585	35·74	1·163	5·646	12·93
Mean	1·128	19·86	23·76	1·131	4·626	95·20
Std. Deviation	0·2860	41·55	24·21	0·1612	5·453	377·3
Std. Error of Mean	0·06394	9·290	5·413	0·03605	1·219	84·38

Table S15. Spearman test for determination of correlation between NtAb to rAds and RBD-specific IgG in participants. The table shows the correlation coefficient (r) and the p-value (p).

			NtAb to rAd26		NtAb to rAd5	
		Day	r	p	r	p
Gam-COVID-Vac	rAd26-S	14	-0·44	0·29	-0·41	0·29
		21	-0·1	0·92	-0·49	0·21
		28	-0·1	0·92	-0·49	0·21
	rAd5-S	14	0·02	>0·99	-0·39	0·31
		21	0·31	0·47	-0·51	0·19
		28	0·35	0·36	-0·43	0·25
	rAd26-S + rAd5-S	14	0·07	0·77	-0·2	0·39
		21	0·36	0·11	-0·01	0·96
		28	0·23	0·33	-0·11	0·65
		42	0·04	0·87	-0·38	0·1
Gam-COVID-Vac-Lyo	rAd26-S	14	-0·27	0·5	-0·07	0·84
		21	0·42	0·25	0·35	0·38
		28	0·53	0·17	0·21	0·59
	rAd5-S	14	-0·57	0·11	-0·57	0·11
		21	-0·56	0·22	-0·56	0·22
		28	-0·49	0·33	-0·49	0·33
	rAd26-S + rAd5-S	14	-0·44	0·06	-0·06	0·8
		21	-0·1	0·69	0·04	0·87
		28	-0·24	0·31	0·24	0·31
		42	-0·26	0·28	0·003	0·99